



COPY OF PAPERS
ORIGINALLY FILED

09902481 .031700 3 45
Box 844

PATENT

Attorney Docket No.: A-70586-1/RFT/RMS/RMK

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

SPRINGER et al.

Serial No. 09/902,481

Filed: July 9, 2001

For: NOVEL PROTEINS WITH
INTEGRIN-LIKE ACTIVITY

Examiner: UNK NOWN

Group Art Unit: 1653

RECEIVED
MAR 19 2002
TECH CENTER 1600/2900

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Assistant Commissioner of Patents, BOX SEQUENCE, Washington, DC 20231 on 3-4-02, 2001.

Signed: Marjorie Jost

Marjorie Jost

PRELIMINARY AMENDMENT RE SEQUENCE LISTING

Assistant Commissioner for Patents
BOX SEQUENCE
Washington, DC 20231

Sir:

This Amendment is in anticipation of a Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence And/or Amino Acid Sequence Disclosures and in compliance with 37 C.F.R. § 1.821-1.825. Although no fee is believed to be due at this time, the Commissioner is authorized to charge any fees including extension fees or other relief which may be required, or credit any overpayment to Deposit Account No. 06-1300 (Our Order No. A-70586-1-1/RFT/RMS/RMK).

Serial No.: 09/902,481

Filed: July 9, 2001

Please amend the application as follows to comply with requirements for patent applications containing nucleotide sequence and/or amino acid sequence disclosures in adherence with rules 37 C.F.R. § 1.821-1.825:

IN THE SPECIFICATION:

Please replace the paragraph beginning at page 5, line 30, with the following rewritten paragraph:

– Figure 1. Stereoview of mutations in the Mac-1 I-domain open structures (active/high affinity/ido). Fig 1A depicts wild type 1ido (open) structure. Fig. 1B depicts the structure computed for the ido1q (open) mutant. Fig 1C depicts the structure computed for the ido1r (open) mutant. Fig 1D depicts the structure computed for the ido2r (open) mutant. Mutant sequences and rotamers were computed as described herein. A cavity was detected in the wild-type 1ido structure but not in the designed mutants, using VOIDOO (Kleywegt et al., Acta Cryst D50:178-185 (1994)) (with a probe of 1.4 Å, a van der Waals growth factor of 1.1, and a minimum of 5 voxels. The cavity is 202 Å³ in 1ido. The cavity is filled by mutations V238F and V160I in ido1q (Fig 1B), V238F and F156W in ido1r (Fig 1C), and V238I in ido2r (Fig 1D). Figure made with Ribbons (Carson, Methods in Enzymology 277:493-505). Fig 1E is a cartoon representation of a complete integrin heterodimer. The black circles represent bivalent cation binding sites. Fig 1F depicts the amino acid sequence of Mac-1 alpha subunit of integrin (SEQ ID NO:1). Fig 1G depicts the nucleotide sequence of Mac-1 alpha subunit of integrin (SEQ ID NO:2). –

Please replace paragraph beginning at page 21, line 28, with the following rewritten paragraph:

– The variant integrin proteins and nucleic acids of the invention are distinguishable from naturally occurring integrins. By “naturally occurring” or “wild type” or grammatical equivalents, herein is meant an amino acid sequence or a nucleotide sequence that is found in nature and includes

Serial No.: 09/902,481

Filed: July 9, 2001

allelic variations; that is, an amino acid sequence or a nucleotide sequence that usually has not been intentionally modified. Accordingly, by "non-naturally occurring" or "synthetic" or "recombinant" or grammatical equivalents thereof, herein is meant an amino acid sequence or a nucleotide sequence that is not found in nature; that is, an amino acid sequence or a nucleotide sequence that usually has been intentionally modified. It is understood that once a recombinant nucleic acid is made and reintroduced into a host cell or organism, it will replicate non-recombinantly, i.e., using the *in vivo* cellular machinery of the host cell rather than *in vitro* manipulations, however, such nucleic acids, once produced recombinantly, although subsequently replicated non-recombinantly, are still considered recombinant for the purpose of the invention. A representative amino acid sequences of a naturally occurring human integrin is shown in Figure 1F (SEQ ID NO:1). It should be noted that unless otherwise stated, all positional numbering of integrin proteins and integrin nucleic acids is based on these sequences (with position 1 equivalent to position 17 of Fig 1F). That is, as will be appreciated by those in the art, an alignment of integrin proteins can be done using standard programs, as is outlined below, with the identification of "equivalent" positions between the two proteins. Thus, the variant integrin proteins and nucleic acids of the invention are non-naturally occurring; that is, they do not exist in nature.—

Please replace paragraph beginning at page 31, line 20, with the following rewritten paragraph:

- In a preferred embodiment the variant integrin proteins of the invention will have a sequence that differs from a wild-type human integrin protein in at least three amino acid position selected from any of the positions in table 1.—

Please replace paragraph beginning at page 31, line 37, with the following rewritten paragraph:

- In a more preferred embodiment, the actual amino acid characteristics of each of the above possible positional mutants is defined in table 1 (SEQ ID NOS:3-6).—

Serial No.: 09/902,481

Filed: July 9, 2001

Please replace paragraph beginning at page 53, line 27, with the following rewritten paragraph:

— In a preferred embodiment, the fusion partner is a stability sequence to confer stability to the library member or the nucleic acid encoding it. Thus, for example, peptides may be stabilized by the incorporation of glycines after the initiation methionine (MG or MGG0), for protection of the peptide to ubiquitination as per Varshavsky's N-End Rule, thus conferring long half-life in the cytoplasm. Similarly, two prolines at the C-terminus impart peptides that are largely resistant to carboxypeptidase action. The presence of two glycines prior to the prolines impart both flexibility and prevent structure initiating events in the di-proline to be propagated into the candidate peptide structure. Thus, preferred stability sequences are as follows: $MG(X)_nGGPP$ (SEQ ID NO:7), where X is any amino acid and n is an integer of at least four.—

Please replace the paragraph beginning at page 72, line 7, with the following rewritten paragraph:

— Four mutant sequences (SEQ ID NOS:3-6) each were computed based on the open 1ido structure and the closed 1jlm structure using two different solvation potentials and subsets of core residues. Three out of a total of four designed ido mutants were well expressed; all have unique amino acid substitutions (Table 1). Fewer substitutions were predicted for jlm mutants, and only one of these, jlm2r (SEQ ID NO:6), was tested. All mutated sidechains are buried in the core of the I domain and are distant from the MIDAS and from the residues critical for iC3b binding (Li, R., et al. J. Cell Biol. 143:1523-1534 (1998); Zhang, L. & Plow, E.F. Biochemistry 38:8064-8071 (1999)), which are located on the top of the I-domain (Figure 1). Thus, the mutated residues cannot directly affect binding of iC3b. The energies of the selected sequences were determined in both the 1ido and 1jlm backbones (Table 1). All of the mutant sequences had energies that were lower than wild-type in the desired backbone configuration, and higher than wild-type in the undesired configuration. Thus, the open ido1q, 1do1r, and ido2r mutants (SEQ ID NOS:3-5) both stabilized the alphaM I domain in the 1ido conformation and destabilized it in the 1jlm

Serial No.: 09/902,481

Filed: July 9, 2001

conformation(Harbury, et al. Science 282:1462-1467 (1998)). Similar results were obtained regardless of the solvation potential used in the calculation. The energy of the wild-type sequence was lower in the 1jlm structure than in the 1ido structure, and thus the wild-type sequence should favor the 1jlm conformation (Table 1). This is consistent with the finding that for all alphaM, alphaL, alpha2 and alpha1 I-domain crystal structures determined to date, the I domain assumes a closed, 1jlm-like structure in the absence of a bound ligand or pseudo-ligand(Lee, et al., Cell 80:631-638 (1995); Qu, A. & Leahy, D.J. Proc. Natl. Acad. Sci. U.S.A. 92:10277-10281 (1995); Qu, A. & Leahy, D.J. Structure 4:931-942 (1996); Emsley, et al., J. Biol. Chem. 272:28512-28517 (1997); Baldwin, E.T. et al. Structure 6:923-935 (1998); Nolte, M. et al. FEBS Lett. 452:379-385 (1999); Rich, R.L. et al. J. Biol. Chem. 274:24906-24913 (1999)).—

On page 75, immediately preceding the heading "CLAIMS", please insert the enclosed text entitled "SEQUENCE LISTING".

REMARKS

The specification has been amended to include a Sequence Listing and proper reference to the sequences therein and to correct minor typographical errors. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

Entry of this amendment is respectfully requested. The amendments are made in adherence with 37 C.F.R. § 1.821-1.825. This amendment is accompanied by a floppy disk containing the above named sequence, SEQUENCE ID NUMBERS1-6 in computer readable form, and a paper copy of the sequence information. The computer readable sequence listing was

Serial No.: 09/902,481

Filed: July 9, 2001

prepared through use of the software program "PatentIn" provided by the PTO. The information contained in the computer readable disk is identical to that of the paper copy. This amendment contains no new matter. Applicant submits that this amendment, the accompanying computer readable sequence listing, and the paper copy thereof serve to place this application in a condition of adherence to the rules 37 C.F.R. § 1.821-1.825.

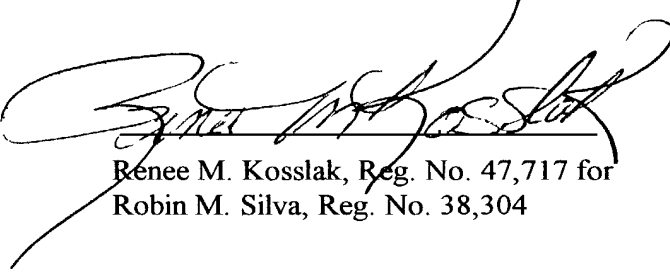
Please direct any calls in connection with this application to the undersigned at (415) 781-1989.

Respectfully submitted,

FLEHR HOHBACH TEST
ALBRITTON & HERBERT LLP

Dated: 3/4/02

Four Embarcadero Center
Suite 3400
San Francisco, CA 94111-4187
Telephone: (415) 781-1989


Renee M. Kossiak, Reg. No. 47,717 for
Robin M. Silva, Reg. No. 38,304

Serial No.: 09/902,481

Filed: July 9, 2001

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Paragraph beginning at page 5, line 30, has been amended as follows:

– Figure 1. Stereoview of mutations in the Mac-1 I-domain open structures (active/high affinity/ido). Fig 1A depicts wild type 1ido (open) structure. Fig. 1B depicts the structure computed for the ido1q (open) mutant. Fig 1C depicts the structure computed for the ido1r (open) mutant. Fig 1D depicts the structure computed for the ido2r (open) mutant. Mutant sequences and rotamers were computed as described herein. A cavity was detected in the wild-type 1ido structure but not in the designed mutants, using VOIDOO (Kleywegt et al., Acta Cryst D50:178-185 (1994)) (with a probe of 1.4 Å, a van der Waals growth factor of 1.1, and a minimum of 5 voxels. The cavity is 202 Å³ in 1ido. The cavity is filled by mutations V238F and V160I in ido1q (Fig 1B), V238F and F156W in ido1r (Fig 1C), and V238I in ido2r (Fig 1D). Figure made with Ribbons (Carson, Methods in Enzymology 277:493-505). Fig 1E is a cartoon representation of a complete integrin heterodimer. The black circles represent bivalent cation binding sites. Fig 1F depicts the amino acid sequence of Mac-1 alpha subunit of integrin (SEQ ID NO:1). Fig 1G depicts the nucleotide sequence of Mac-1 alpha subunit of integrin (SEQ ID NO:2). –

Paragraph beginning at page 21, line 28, has been amended as follows:

– The variant integrin proteins and nucleic acids of the invention are distinguishable from naturally occurring integrins. By “naturally occurring” or “wild type” or grammatical equivalents, herein is meant an amino acid sequence or a nucleotide sequence that is found in nature and includes allelic variations; that is, an amino acid sequence or a nucleotide sequence that usually has not been intentionally modified. Accordingly, by “non-naturally occurring” or “synthetic” or

Serial No.: 09/902,481

Filed: July 9, 2001

"recombinant" or grammatical equivalents thereof, herein is meant an amino acid sequence or a nucleotide sequence that is not found in nature; that is, an amino acid sequence or a nucleotide sequence that usually has been intentionally modified. It is understood that once a recombinant nucleic acid is made and reintroduced into a host cell or organism, it will replicate non-recombinantly, i.e., using the *in vivo* cellular machinery of the host cell rather than *in vitro* manipulations, however, such nucleic acids, once produced recombinantly, although subsequently replicated non-recombinantly, are still considered recombinant for the purpose of the invention. A representative amino acid sequences of a naturally occurring human integrin is shown in Figure 1F (SEQ ID NO:1). It should be noted that unless otherwise stated, all positional numbering of integrin proteins and integrin nucleic acids is based on these sequences (with position 1 equivalent to position 17 of Fig 1F). That is, as will be appreciated by those in the art, an alignment of integrin proteins can be done using standard programs, as is outlined below, with the identification of "equivalent" positions between the two proteins. Thus, the variant integrin proteins and nucleic acids of the invention are non-naturally occurring; that is, they do not exist in nature.—

Paragraph beginning at page 31, line 20, has been amended as follows:

- In a preferred embodiment the variant integrin proteins of the invention will have a sequence that differs from a wild-type human integrin protein in at least three (~~Dr Springer, do you know of an integrin construct with more than 3 mutations apart from chimeras???~~) amino acid position selected from any of the positions in table 1.—

Paragraph beginning at page 31, line 37, has been amended as follows:

- In a more preferred embodiment, the actual amino acid characteristics of each of the above possible positional mutants is defined in table 1 (SEQ ID NOS:3-6).—

Serial No.: 09/902,481

Filed: July 9, 2001

Paragraph beginning at page 53, line 27, has been amended as follows:

— In a preferred embodiment, the fusion partner is a stability sequence to confer stability to the library member or the nucleic acid encoding it. Thus, for example, peptides may be stabilized by the incorporation of glycines after the initiation methionine (MG or MGG0), for protection of the peptide to ubiquitination as per Varshavsky's N-End Rule, thus conferring long half-life in the cytoplasm. Similarly, two prolines at the C-terminus impart peptides that are largely resistant to carboxypeptidase action. The presence of two glycines prior to the prolines impart both flexibility and prevent structure initiating events in the di-proline to be propagated into the candidate peptide structure. Thus, preferred stability sequences are as follows: $MG(X)_nGGPP$ (SEQ ID:NO:7), where X is any amino acid and n is an integer of at least four.—

Paragraph beginning at page 72, line 7, has been amended as follows:

— Four mutant sequences (SEQ ID NOS:3-6) each were computed based on the open 1ido structure and the closed 1jlm structure using two different solvation potentials and subsets of core residues. Three out of a total of four designed ido mutants were well expressed; all have unique amino acid substitutions (Table 1). Fewer substitutions were predicted for jlm mutants, and only one of these, jlm2r (SEQ ID NO:6), was tested. All mutated sidechains are buried in the core of the I domain and are distant from the MIDAS and from the residues critical for iC3b binding (Li, R., et al. J. Cell Biol. 143:1523-1534 (1998); Zhang, L. & Plow, E.F. Biochemistry 38:8064-8071 (1999)), which are located on the top of the I-domain (Figure 1). Thus, the mutated residues cannot directly affect binding of iC3b. The energies of the selected sequences were determined in both the 1ido and 1jlm backbones (Table 1). All of the mutant sequences had energies that were lower than wild-type in the desired backbone configuration, and higher than wild-type in the undesired configuration. Thus, the open ido1q, 1do1r, and ido2r mutants (SEQ ID NOS:3-5) both stabilized the alphaM I domain in the 1ido conformation and destabilized it in the 1jlm

Serial No.: 09/902,481

Filed: July 9, 2001

conformation(Harbury, et al. Science 282:1462-1467 (1998)). Similar results were obtained regardless of the solvation potential used in the calculation. The energy of the wild-type sequence was lower in the 1jlm structure than in the 1ido structure, and thus the wild-type sequence should favor the 1jlm conformation (Table 1). This is consistent with the finding that for all alphaM, alphaL, alpha2 and alpha1 I-domain crystal structures determined to date, the I domain assumes a closed, 1jlm-like structure in the absence of a bound ligand or pseudo-ligand(Lee, et al., Cell 80:631-638 (1995); Qu, A. & Leahy, D.J. Proc. Natl. Acad. Sci. U.S.A. 92:10277-10281 (1995); Qu, A. & Leahy, D.J. Structure 4:931-942 (1996); Emsley, et al., J. Biol. Chem. 272:28512-28517 (1997); Baldwin, E.T. et al. Structure 6:923-935 (1998); Nolte, M. et al. FEBS Lett. 452:379-385 (1999); Rich, R.L. et al. J. Biol. Chem. 274:24906-24913 (1999)).—

On page 75, immediately preceding the claims, the enclosed Sequence Listing was added to the text.